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CERTIFICATION REPORT

The certification of the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in surface water: ERM®-CA100

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Abstract
This report describes the production of ERM®-CA100, a surface water material certified for the concentrations of naphthalene, anthracene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene. The material was produced following ISO Guide 34:2009.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 .
The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in compliance with the ISO/IEC Guide 98-3 "Guide to the Expression of Uncertainty in Measurement (GUM:1995)" ; the total estimated uncertainty includes uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for use in the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in a unit kit that includes a plastic container with at least 1000 mL of surface water, an amber glass ampoule with at least 24 mL humic acids solution and an amber glass ampoule with at least 2 mL polycyclic aromatic hydrocarbons solution. A reconstitution protocol must be strictly followed to obtain the final CRM.
The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

CERTIFICATION REPORT

The certification of the concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in surface water: ERM®-CA100

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM®-CA100, a surface water material certified for the concentrations of naphthalene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene. The material was produced following ISO Guide 34:2009 [1].

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2].

The material was characterised by an intercomparison among laboratories of demonstrated competence and adhering to ISO/IEC 17025 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in compliance with the ISO/IEC Guide 98-3 “Guide to the Expression of Uncertainty in Measurement (GUM:1995)” [4]; the total estimated uncertainty includes uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for use in the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The CRM is available in a unit kit that includes a plastic container with at least 1000 mL of surface water, an amber glass ampoule with at least 24 mL humic acids solution and an amber glass ampoule with at least 2 mL polycyclic aromatic hydrocarbons solution. A reconstitution protocol must be strictly followed to obtain the final CRM.

The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

The following values were assigned:

	Mass concentration	
	Certified value [1)]	Uncertainty [2)]
Naphthalene	1.21 µg/L	0.13 µg/L
Anthracene	91 ng/L	11 ng/L
Fluoranthene	104 ng/L	11 ng/L
Benzo[*b*]fluoranthene	32 ng/L	9 ng/L
Benzo[*k*]fluoranthene	38 ng/L	9 ng/L
Benzo[*a*]pyrene	42 ng/L	8 ng/L
Indeno[1,2,3-*cd*]pyrene	29 ng/L	7 ng/L

1) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination after sample reconstitution strictly following the protocol specified in the “Instructions for use”. The certified value and its uncertainty are traceable to the International System of units (SI).

2) The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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Glossary

ANOVA	Analysis of variance
c	Mass concentration $c = m / V$ (mass / volume)
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CRM	Certified reference material
EC	European Commission
EQS	Environmental Quality Standard(s)
ERM®	Trademark of European Reference Materials
EU	European Union
GC-MS	Gas chromatography-mass spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements
HA	Humic acids
HPLC	High performance liquid chromatography
HPLC-FLD	High performance liquid chromatography-fluorescence detection
i	Result position in the analytical sequence
IEC	International Electrotechnical Commission
IRMM	Institute for Reference Materials and Measurements of the JRC
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated
PAH	Polycyclic aromatic hydrocarbons
PS	Priority substances
QA	Quality assurance
QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material

s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of units
s_{meas}	Standard deviation of measurement data; an additional index "rel" is added as appropriate
SPE	Solid phase extraction
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
$t_{\alpha, df}$	Critical t-value for a t-test, with a level of confidence of 1-α and df degrees of freedom
TOC	Total organic carbon
t_{sl}	Proposed shelf life
t_{tt}	Transport time chosen
u	Standard uncertainty
U	Expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_{Δ}	Combined standard uncertainty of measurement result and certified value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel"

	is added as appropriate
u_t	Standard uncertainty of trueness
WFD	Water Framework Directive
$\bar{x}$	Arithmetic mean
α	Significance level
Δ_{meas}	Absolute difference between mean measured value and the certified value

1 Introduction

1.1 Background

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of organic substances with two or more benzene rings in their structure, originating mainly from human activities (i.e., fossil fuel combustion) but also from natural events (volcanos and fires) [5]. They are ubiquitous in the environment and due to the wide range of molecular masses of the analytes in this group their physical and chemical properties vary, as well as their effects. In general, PAHs are hydrophobic, which enhances their affinity for organic matter and suspended particulate matter in aquatic environments, leading to their adsorption and sedimentation. The lower mass analytes exhibit acute toxicity for aquatic organisms, while higher mass analytes possess carcinogenic or mutagenic properties; the affinity of PAHs to lipids contributes to the delivery of these pollutants to aquatic organisms [6].

Since 2000, Directive 2000/60/EC, known as the Water Framework Directive (WFD) [7] is in force in the European Union (EU) for developing a pollution control strategy of all EU water bodies. Subsequently, Directive 2008/105/EC [8] set Environmental Quality Standards (EQS) for the established list of Priority Substances (PS). PAHs are among the PS that Member States are expected to assess, monitor and control in EU water resources. The most recent Directive 2013/39/EU [9] re-evaluated the EQS for some of the PS including PAHs, enhancing the analytical challenges for the monitoring laboratories by lowering even more the concentration levels.

To ensure the quality and comparability of analytical results reported by the Member States, the EU has issued Directive 2009/90/EC [10] in 2009, setting minimum analytical performance criteria for monitoring water quality. The competence of EU laboratories selected for these tasks must be guaranteed by the use of certified reference materials (CRMs) of appropriate matrix and with corresponding levels to the established EQS.

1.2 Choice of the material

In support of the EU legislation, ERM-CA100 was developed as a natural water matrix CRM, where humic acids (HA) are added via a reconstitution protocol to simulate organic matter, according to the requirements of the WFD. The design of this CRM addresses as close as possible the specific legislative request of the WFD for “whole water sample” analysis and the addition of the HA targets the adsorption issues of the 8 PAHs that are regulated by the WFD: naphthalene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene.

The PAH concentrations in the designed CRM were established either at EQS levels in force when the project initiated [8] or as close to the EQS as possible in the event that these levels were not realistically achievable due to technical limitations in the analysis. With the enforcement of Directive 2013/39/EU [9] the EQS values were changed to significantly lower levels for fluoranthene and benzo[*a*]pyrene; additionally, benzo[*a*]pyrene is now considered as a marker for benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene, which do not need to be monitored any more.

1.3 Design of the project

The material is designed as a unit box set that contains three items (a water sample bottle, a HA ampoule and a PAH ampoule). A reconstitution protocol needs to be strictly followed to obtain the CRM. Homogeneity and stability were assessed for all PAHs in the final reconstituted material. The characterisation was performed by interlaboratory comparison using different analytical methods to reduce bias in the analytical result.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

IVL Svenska Miljöinstitutet, Swedish Environmental Research Institute Ltd, Stockholm, SE
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; 1213)

2.4 Stability study

IVL Svenska Miljöinstitutet, Swedish Environmental Research Institute Ltd, Stockholm, SE
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; 1213)

GBA, Gesellschaft für Bioanalytik mbH, Pinneberg, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS; D-PL-14170-01-00)

2.5 Characterisation

BAM, Bundesanstalt für Materialforschung und –prüfung, Berlin, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS; D-PL-11075-14-00)

GBA, Gesellschaft für Bioanalytik mbH, Pinneberg, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS; D-PL-14170-01-00)

Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS; D-PL-14138-02-00)

INERIS, Institut National de l'Environnement Industriel et des Risques, Verneuil-En-Halatte, FR

IVL Svenska Miljöinstitutet, Swedish Environmental Research Institute Ltd, Stockholm, SE
(measurements under the scope of ISO/IEC 17025 accreditation SWEDAC; 1213)

NCSR Demokritos, National Center for Scientific Research, Athens, GR
(measurements under the scope of ISO/IEC 17025 accreditation E.SY.D.; 580-2)

Rijkswaterstaat - Institute for Inland Water Management and Waste Water Treatment RIZA, Lelystad, NL
(measurements under the scope of ISO/IEC 17025 accreditation Raad voor Accreditatie/Dutch Accreditation Council; L521)

SGS Belgium NV, NK–'s-Gravenpolder, NL
(measurements under the scope of ISO/IEC 17025 accreditation BELAC; 005-TEST)

TNO, Earth, Life & Social Sciences (ELSS), Utrecht, NL
(measurements under the scope of ISO/IEC 17025 accreditation Raad voor Accreditatie/Dutch Accreditation Council; L026)

Umweltbundesamt GmbH, Wien, AU
(measurements under the scope of ISO/IEC 17025 accreditation BMWFJ; 0200)

VITO NV, Vlaamse Instelling voor Technologisch Onderzoek, Mol, BE

VMM Vlaamse Milieumaatschappij, Sint-Denijs-Westrem, BE
(measurements under the scope of ISO/IEC 17025 accreditation BELAC; 163-TEST)

3 Material processing and process control

3.1 Origin of the starting material

The starting material used for the production of ERM-CA100 was surface water originating from the premises of the Institute for Reference Materials and Measurements, Retieseweg 111, Geel, Belgium. The sampling took place on the 5th of October 2010 and the water was collected from a pond within IRMM premises. The amount collected was 1200 L of surface water in pre-cleaned high density polyethylene drums of 1000 L and 200 L. Six 5 µm pore size membrane filters were used for initial filtration of the water, approximately one filter every 200 L of water. The pump used was a Watson Marlow model 624U peristaltic pump (Falmouth, UK).

Humic acids were purchased by Sigma-Aldrich N.V (technical grade with ~ 20 % residue impurities)

PAHs were purchased as neat crystals as follows:

Naphthalene and benzo[*ghi*]perylene by Fluka (99.9 % and 98.0 %, respectively)

Anthracene and indeno[1,2,3-*cd*]pyrene by Dr. Ehrenstorfer (99.5 % and 99.0 %, respectively)

Fluoranthene by Sigma-Aldrich N.V. (99.6 %)

Benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and benzo[*a*]pyrene by Supelco (99.9 %, 99.9 % and 99.2 %, respectively)

3.2 Processing

Flowcharts of the complete production process are presented below in Figures 1 – 3.

The containers of 1000 L and 200 L with the starting material were stored at 4 °C and after further sedimentation and stabilisation, the material was transferred through filters [Acropak 1000 Capsule of 0.8/0.2 µm (Pall)] to 3 pre-cleaned vessels (400 L water each).

Before initiating the processing, blank levels for the target PAHs were analysed in the filtered water, the containers to be used for the production of the CRM, as well as in the HA solution.

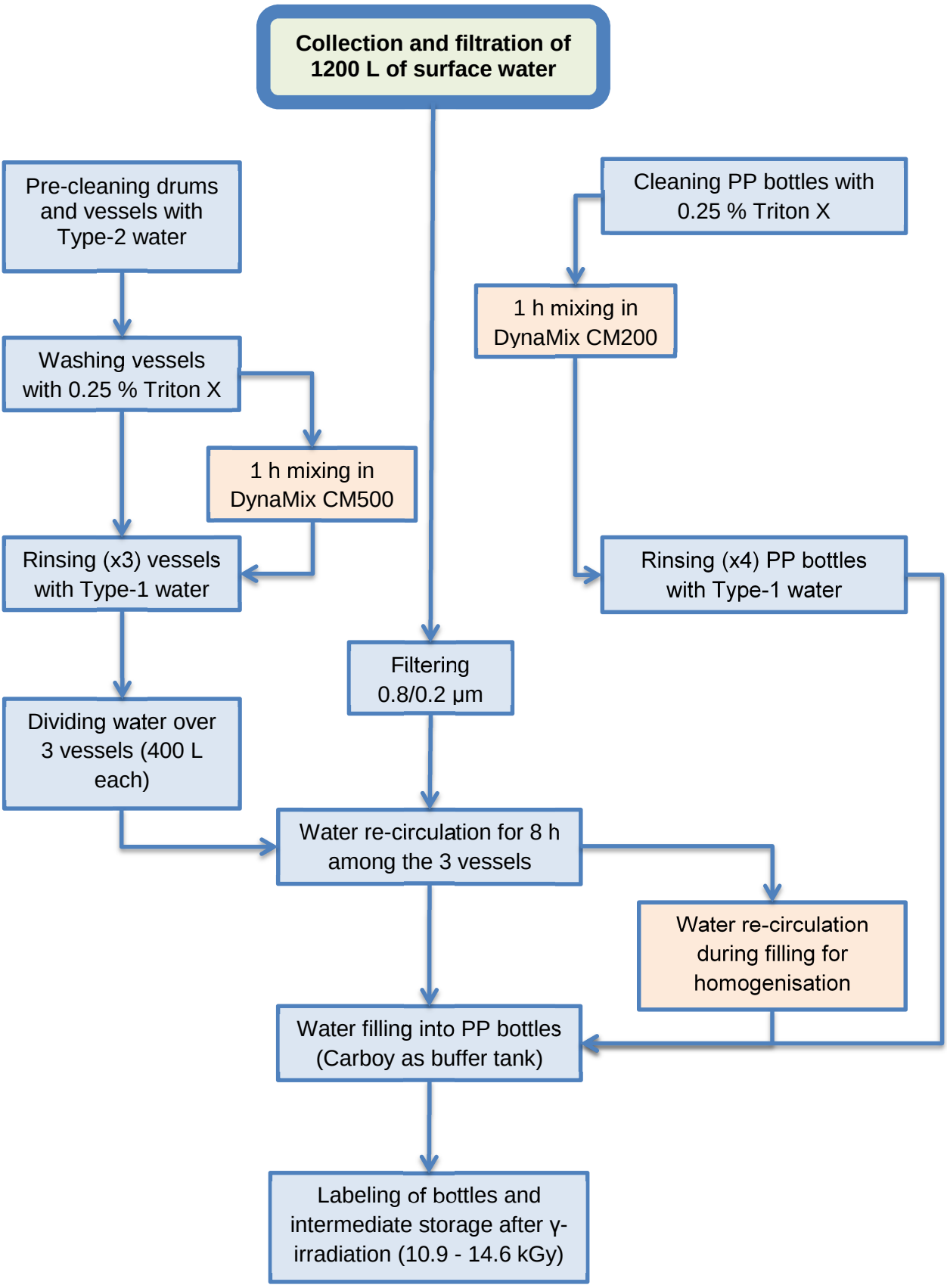

Figure 1: Production processing of water bottles

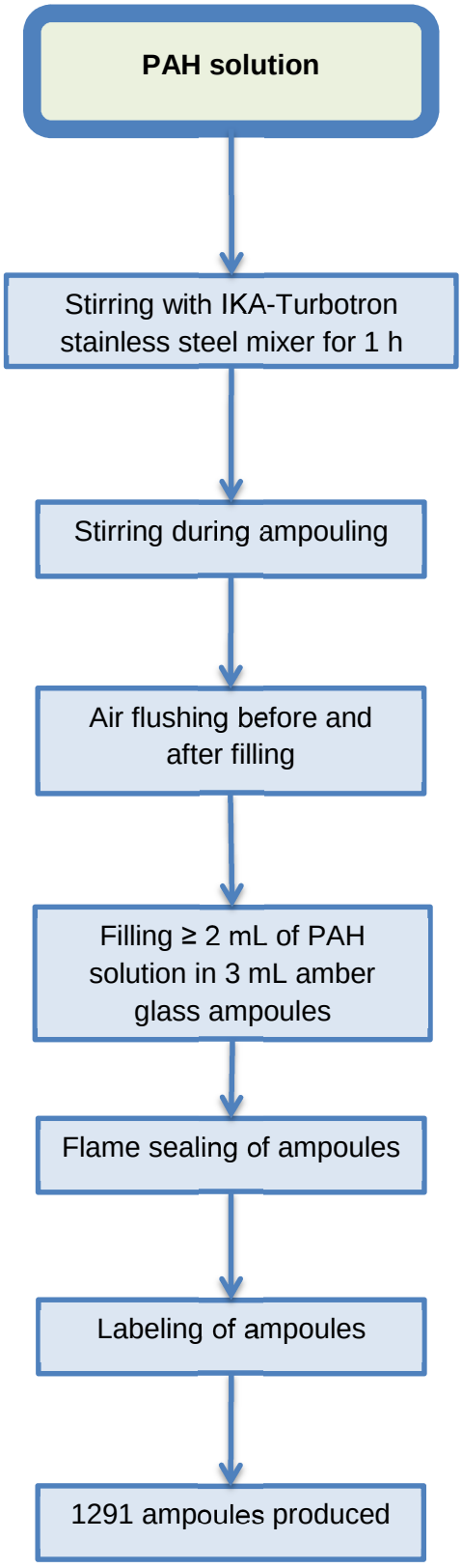

Figure 2: Production processing of PAH spiking solution ampoules

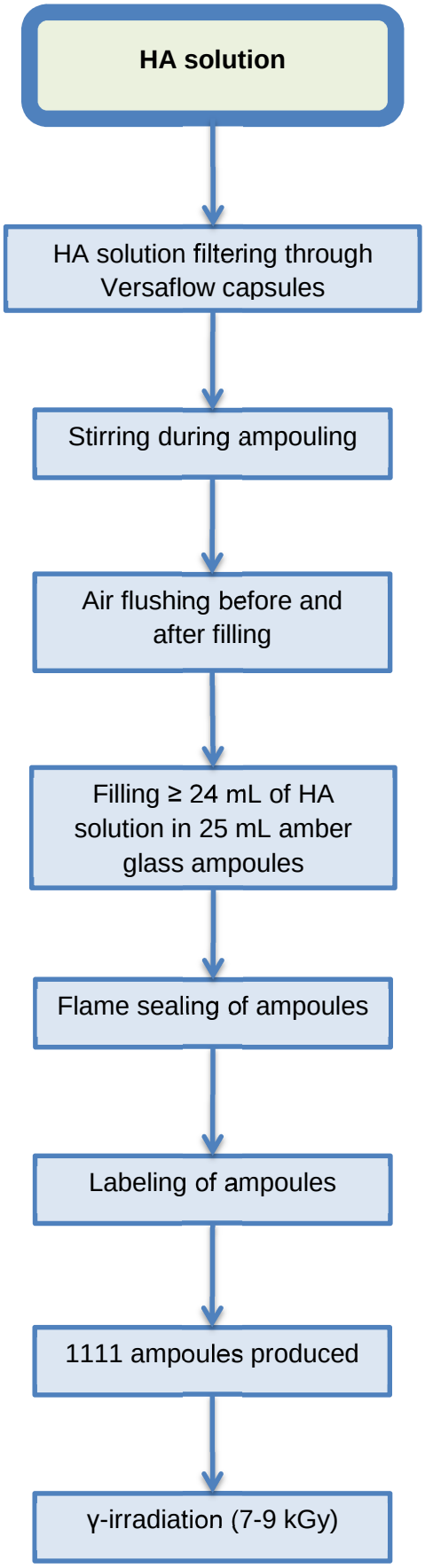

Figure 3: Production processing of HA spiking solution ampoules

3.2.1 Water sample bottles

All vessels, containers and sample bottles were carefully cleaned according to established protocols and using a 3-dimensional mixer (Dyna-MIX CM500 system, WAB, Basel, CH) and 0.25 % (v/v) Triton X, as well as manual rinsing steps with MilliQ water. All 1000 mL polypropylene bottles with leak-proof corkscrews were obtained from VWR (Haasrode, BE).

All parts in contact with the water (pumps, tubing, connections) were made of inert perfluoroalkyoxy-polymers or other fluoro-polymers and they were carefully cleaned with Triton X and rinsed with MilliQ water as well [IWAKI FS-30HT2 inert bellow pumps (Tokyo, JP)].

The filling of the sample bottles followed the scheme below (Figure 4):

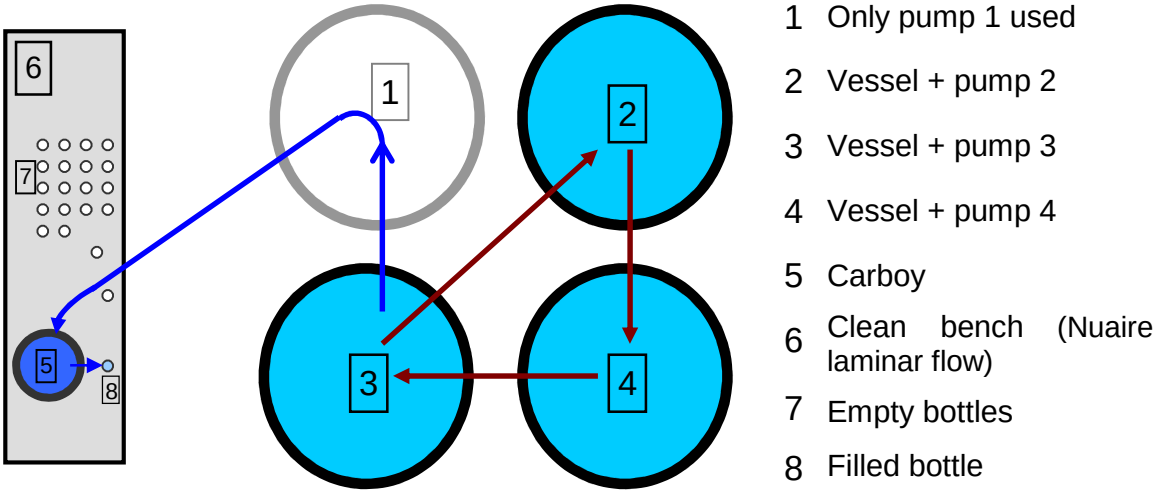

Figure 4: During filling, the water was re-circulated between vessels 2, 3 & 4 to ensure homogenisation whereas the bellow pump of drum 1 was used to transfer the water from drum 3 into the Carboy, a smaller vessel equipped with a tap. The bottles were filled by gravity from the Carboy's tap.

The bottles were filled with water volumes between 1010 and 1060 mL, weighed for checking the filled volume, closed with a torque wrench, labeled and stored in cardboard boxes. The filling was performed in a clean bench which had previously been checked to provide a dust-free environment. In total 1111 bottles were filled.

The cardboard boxes with the water bottles were placed on three pallets and sent for γ-irradiation in order to sterilise the water (Synergy Health, Etten-Leur, NL). A dose between 10.9 - 14.6 kGy was applied to the samples.

3.2.2 PAH ampoules

Blank checks of ampoules from NAF (Nijmegen, NL) were performed using gas chromatography coupled with mass spectrometry (GC-MS). The acetonitrile (Reag Ph EUR, Merck KGaA, Darmstadt, DE) used as solvent was analysed by GC-MS direct injection. Based on the results of the analyses, which were lower than the limits of detection (LOD), possible PAH contamination was excluded. The PAH spiking solution was prepared by metrological weighing and further diluted volumetrically in acetonitrile; the theoretical concentration values obtained as average of five independently prepared solutions are shown in Table 1 (these values were additionally confirmed by in-house GC-MS measurements). A Rota ampouling machine R 910 PA (Rota, Wehr, DE) was set up to fill at least 2.3 mL of the PAH solution in amber glass ampoules. The PAH solution was under constant magnetic stirring during filling for homogenisation purposes (Figure 2). For every

ampoule a continuous visual check that the PAH solution did not splash inside the neck of the ampoule (possibly causing in situ soot formation that may contain PAHs) was performed. In this way 1291 units of PAH solution (solution B of ERM-CA100) were produced.

Table 1: Theoretical values of the PAHs in the spiking solution (solution B)

Analyte	Theoretical concentration [ng/mL]
Naphthalene	602.51
Anthracene	48.65
Fluoranthene	50.65
Benzo[*b*]fluoranthene	17.61
Benzo[*k*]fluoranthene	20.16
Benzo[*a*]pyrene	25.53
Indeno[1,2,3-*cd*]pyrene	19.71
Benzo[*ghi*]perylene	22.30

3.2.3 Humic acids ampoules

The solid humic acids were dissolved in Type-1 water [11] using an ultrasonic bath and was centrifuged and pooled in two pre-cleaned plastic drums containing 18 and 24.5 L, respectively. A blank check for PAHs was done on the bulk humic acid solution. Based on the results of the analyses, which were lower than the LOD, possible PAH contamination was excluded. The produced HA solutions were filtered through 0.8/0.45 µm membrane filters (Pall, Versaflow capsule P/N 12131, Farlington, UK). In all instances a Watson Marlow 624U peristaltic pump (Falmouth, UK) was used for filtering. Prior to filtering, the vessels with the HA solution had also been left standing over night as to allow sedimentation. The HA solution was stirred with an IKA mixer (Staufen, DE) for 30 minutes before starting the filling. A Rota ampouling machine R 910 PA (Rota, Wehr, DE) was set up to fill at least 24 mL of the HA solution in amber glass ampoules. The solution was constantly under stirring with a stainless steel paddle during the filling and care was taken to avoid foaming (Figure 3). Filling of 1111 ampoules of HA solution (solution A of ERM-CA100) was achieved. The ampoules were sent for γ-irradiation (Synergy-Health, Etten-Leur, NL) after being temporarily placed in plastic tubes for safety reasons and protection of the ampoules during irradiation and transport. A dose between 7.2 – 9.0 kGy was applied to the samples.

Total organic carbon (TOC) measurements were conducted in triplicate on 12 ampoules yielding the result of 678 ± 8 mg carbon/L (average of all measurements ± standard deviation).

3.2.4 Final CRM unit

The CRM was produced as a styrofoam box unit kit that includes a plastic container with at least 1000 mL of surface water, an amber glass ampoule with at least 24 mL HA solution in water (labelled as solution A) and an amber glass ampoule with at least 2 mL PAH solution in acetonitrile (labelled as solution B).

The final unit produced can be seen in Figure 5 below:

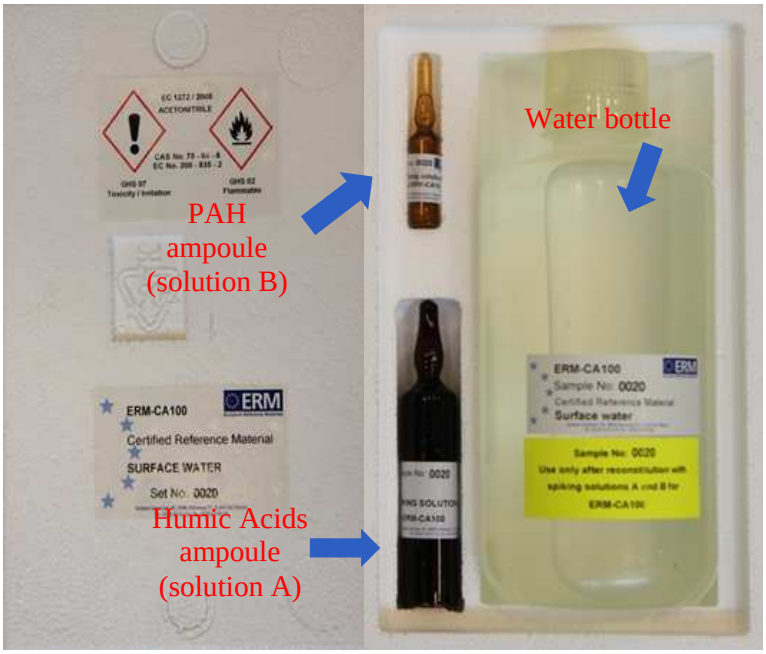

Figure 5: Final ERM-CA100 unit

4 Homogeneity

A key requirement for any reference material is the equivalence between the various units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value. In contrast to that it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34:2009 [1] requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. In the present project, quantification of within-unit inhomogeneity is not necessary, because the minimum sample intake is prescribed by the protocol.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all units of the material, within the stated uncertainty.

Initially, homogeneity of the PAH ampoules and the HA ampoules (the latter via TOC analysis) was assessed and no inhomogeneity was found. The evaluation of the homogeneity of the CRM (after reconstitution) was performed by using the data of the short term stability study. The number of selected units corresponds to approximately the cubic root of the total number of the produced units. Fourteen CRM units were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into seven groups (with a similar number of units in each group) and two units were selected randomly from each group. In the case of anthracene, only seven CRM units were used, given the trend observed for the short term stability study at 18 ºC. Two independent samples were analysed from each selected unit for

the CRM; the samples were analysed by liquid/liquid extraction (LLE) after addition of internal standard and clean-up via a silica gel column. Quantification was performed with high performance liquid chromatography – fluorescence detection (HPLC-FLD). The results of the measurements are shown in Annex A. The measurements were performed under repeatability conditions and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. A significant trend (95 % confidence level) in the analytical sequence was visible for anthracene, pointing at a signal drift in the analytical system. A significant trend (95 % confidence level) in the filling sequence was detected for benzo[*a*]pyrene. The correction of biases, even if they are statistically not significant, was found to combine the smallest uncertainty with the highest probability to cover the true value [12]. Correction of trends is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking potential between-unit heterogeneities. As the analytical sequence and the unit numbers were not correlated, trends significant on at least a 95 % confidence level were corrected as shown below:

$$corrected\ \ result = measured\ \ \ result - b \cdot i \qquad \textbf{Equation 1}$$

b **=** slope of the linear regression

i **=** position of the result in the analytical sequence

All datasets were tested for consistency using Grubbs outlier tests on a confidence level of 99 % on the individual results and the unit means. Outlying individual results were not detected; however, two outlying unit means were detected for benzo[*ghi*]perylene.

Quantification of between-unit inhomogeneity was accomplished by analysis of variance (ANOVA), which can separate the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole unit.

Evaluation by ANOVA requires unit means which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. Distribution of the unit means was visually tested using histograms and normal probability plots. Too few data are available for the unit means to make a clear statement of the distribution. Therefore, it was visually checked whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Table 2.
One has to bear in mind that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean square within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [13]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Table 2: Results of the statistical evaluation of the homogeneity studies

Analyte	Trend (before correction) (95 % confidence level)		Outliers (99 % confidence level)		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
Naphthalene	No	No	None	None	unimodal	unimodal
Anthracene	Yes	No	None	None	unimodal	unimodal
Fluoranthene	No	No	None	None	unimodal	unimodal
Benzo[*b*]fluoranthene	No	No	None	None	unimodal	unimodal
Benzo[*k*]fluoranthene	No	No	None	None	unimodal	unimodal
Benzo[*a*]pyrene	No	Yes	None	None	unimodal	unimodal
Indeno[1,2,3-*cd*]pyrene	No	No	None	None	unimodal	unimodal
Benzo[*ghi*]perylene	No	No	None	Two	unimodal	unimodal

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}} \qquad \textbf{Equation 2}$$

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}} \qquad \textbf{Equation 3}$$

$$u^*_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}}\sqrt[4]{\frac{2}{v_{MSwithin}}}}{\bar{y}} \qquad \textbf{Equation 4}$$

MS_{within} mean square within a unit from an ANOVA

$MS_{between}$ mean squares between-unit from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n number of replicates per unit

$v_{MSwithin}$ degrees of freedom of MS_{within}

However, a different approach was adopted for benzo[*ghi*]perylene for which two outlying unit means were detected. In this case between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean, and the rectangular standard uncertainty of homogeneity was estimated by:

$$u_{rec,rel} = \frac{\left|outlier - \bar{y}\right|}{\sqrt{3} \cdot \bar{y}}$$ **Equation 5**

$\bar{y}$ mean of all results of the homogeneity study

When a trend in the filling sequence was significant at least at 95 % confidence level, the uncertainty was assessed in a different way. This applies for benzo[*a*]pyrene for which u_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean. The corrected uncertainty in the case where there was a significant trend in the filling sequence is given in:

$$u_{rec,rel} = \frac{\left|highest\ result - lowest\ result\right|}{2 \cdot \sqrt{3} \cdot \bar{y}}$$ **Equation 6**

The results of the evaluation of the between-unit variation are summarised in Table 3.

Table 3: Results of the homogeneity study

Analyte	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
Naphthalene	6.5	n.c.	2.8	n.a.	2.8
Anthracene	2.9	2.7	1.5	n.a.	2.7
Fluoranthene	3.0	1.7	1.3	n.a.	1.7
Benzo[*b*]fluoranthene	10.8	n.c.	4.7	n.a.	4.7
Benzo[*k*]fluoranthene	4.3	n.c.	1.9	n.a.	1.9
Benzo[*a*]pyrene	9.0	n.c.	3.9	4.2	4.2
Indeno[1,2,3-*cd*]pyrene	7.7	n.c.	3.3	n.a.	3.3
Benzo[*ghi*]perylene	9.4	n.c.	4.1	7.2	7.2

[1] n.c.: cannot be calculated as $MS_{between} < MS_{within}$
[2] n.a.: not applicable

The homogeneity study showed two outlying unit means for benzo[*ghi*]perylene and a trend in the filling sequence for benzo[*a*]pyrene. However, taking these extreme values into account, the inhomogeneity quantified as u_{rec} is still sufficiently small to make the material useful. Therefore, u_{rec} was used as estimate of u_{bb}. A trend in the analytical sequence for anthracene was corrected. As u^*_{bb} sets the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the certified value within its stated uncertainty.

The minimum sample intake for this CRM is prescribed by the reconstitution protocol and is equal to 500 mL (see Section 9 Instructions for use).

5 Stability

Time, temperature, and radiation were regarded as the most relevant influences on stability of the materials. The influence of ultraviolet or visible radiation was minimised by the choice of the containment which eliminates most of the incoming light for the HA and the PAH solutions. Materials are stored and dispatched in the dark, thus eliminating practically the possibility of degradation by UV and visible radiation. Additionally the material was sterilized by γ-irradiation to eliminate microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as conditions for dispatch to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if transport at ambient temperature will be applied.

The stability studies were carried out using an isochronous design [14]. In that approach, samples are stored for a certain time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples are analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1 Short-term stability study

For the short-term stability studies, samples were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to 4 °C. Two units per storage time were selected (except the two-week time point at 18 °C where three units were chosen and one-week time point at 60 °C where one unit was chosen) using a random stratified sampling scheme, resulting in 14 units in total. From each unit, two samples were measured; the samples were analysed by LLE after addition of internal standard and clean-up via a silica gel column. Quantification was performed with HPLC-FLD. The measurements were performed under repeatability conditions and in a randomised sequence to be able to separate a potential analytical drift from a trend over storage time.

The obtained data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test (99 % confidence level). Two outlying individual results at 18 °C were found for benzo[*k*]fluoranthene (Table 4). As no technical reason for the outliers could be found all data were retained for statistical analysis.

Furthermore, the data were evaluated against storage time and regression lines of concentration versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to shipping conditions). For all elements except anthracene, the slopes of the regression lines were not significantly different from zero (on 95 % confidence level) at both 18 °C and 60 °C. Anthracene exhibited a significant trend only at 18 °C.

The results of the measurements are shown in Annex B. The results of the statistical evaluation of the short-term stability are summarised in Table 4.

Table 4: Results of the short-term stability tests

Analyte	Number of individual outlying results		Significance of the trend on a 95 % confidence level	
	18 ºC	60 ºC	18 ºC	60 ºC
Naphthalene	None	None	No	No
Anthracene	None	None	Yes	No
Fluoranthene	None	None	No	No
Benzo[*b*]fluoranthene	None	None	No	No
Benzo[*k*]fluoranthene	Two	None	No	No
Benzo[*a*]pyrene	None	None	No	No
Indeno[1,2,3-*cd*]pyrene	None	None	No	No
Benzo[*ghi*]perylene	None	None	No	No

The statistical outliers which were detected for benzo[*k*]fluoranthene were retained for the estimation of u_{sts}. A significant trend at 18 °C was found for anthracene, but the material appeared to be stable at 60 °C. As it is unlikely that the material degrades faster at a lower temperature than at a higher one, this was regarded as artefact.

The material can be dispatched without further precautions under ambient conditions.

5.2 Long-term stability study

For the long-term stability study, 32 units were stored at 18 °C for 0, 6, 12 and 18 months. The reference temperature was set to 4 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, two samples were measured; the samples were analysed by LLE after addition of internal standard and clean-up via a silica gel column. Quantification was performed with GC-MS. The measurements were performed under repeatability conditions, in a random sequence to be able to separate any potential analytical drift from a trend over storage time.

The results were screened for outliers using the single and double Grubbs test (99 % confidence level). No outlying individual results were found (Table 5).

Furthermore, the data were plotted against storage time and linear regression lines of concentration versus time were calculated. The slope of the regression lines was tested for statistical significance (loss/increase due to storage conditions). For all analytes except benzo[*b*]fluoranthene and benzo[*k*]fluoranthene, the slopes of the regression lines were not significantly different from zero (on 95 % confidence level) at 18 °C.

The results of the long term stability measurements are shown in Annex C. The results of the statistical evaluation of the long-term stability study are summarised in Table 5.

No technically unexplained outliers were observed. Positive trends were observed for benzo[*b*]fluoranthene and benzo[*k*]fluoranthene at 18 °C. As the analyte in both cases cannot be created in the sample, a positive trend could only be due to degradation of the matrix. This, however, should be seen for all measurands, which is not the case. The observed trend was therefore regarded as an artefact. Nevertheless, degradation was included in the estimation of u_{lts} for these analytes for conservative reasons and the material can be stored at 18 °C. The u_{lts} might be revised in the future on the basis of post-certification measurements.

Table 5: Results of the long-term stability tests (18 ºC)

Analyte	Number of individual outlying results	Significance of the trend on a 95 % confidence level
Naphthalene	None	No
Anthracene	None	No
Fluoranthene	None	No
Benzo[*b*]fluoranthene	None	Yes
Benzo[*k*]fluoranthene	None	Yes
Benzo[*a*]pyrene	None	No
Indeno[1,2,3-*cd*]pyrene	None	No
Benzo[*ghi*]perylene	None	No

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can rule out degradation of materials completely, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability, i.e. to estimate the uncertainty of stability. This means, even under ideal conditions, the outcome of a stability study can only be "degradation is 0 ± x % per time".

Uncertainties of stability during dispatch and storage were estimated as described in [15] for each analyte. For this approach, the uncertainty of the linear regression line with a slope of zero is calculated. The uncertainty contributions u_{sts} and u_{lts} are calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{tt} \qquad \textbf{Equation 7}$$

$$u_{lts,rel} = \frac{RSD}{\sqrt{\sum (t_i - \bar{t})^2}} \cdot t_{sl} \qquad \textbf{Equation 8}$$

RSD	relative standard deviation of all results of the stability study
t_i	time elapsed at time point *i*
$\bar{t}$	mean of all t_i
t_{tt}	chosen transport time (1 week at 60 ºC)
t_{sl}	chosen shelf life (24 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 18 °C and 60 °C studies. The uncertainty describes the possible change during a dispatch at either temperatures lasting for one week.

- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C studies and describes the possible degradation during 24 months storage at 18 °C.

The results of these evaluations are summarised in Table 6.

Table 6: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for the temperatures of 18 °C and 60 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 24 months

Analyte	$u_{sts,rel}$ [%]		$u_{lts,rel}$ [%]
	18 °C	60 °C	18 °C
Naphthalene	1.0	1.0	1.4
Anthracene	0.9	0.8	1.8
Fluoranthene	0.7	0.5	2.6
Benzo[*b*]fluoranthene	1.7	2.0	8.0
Benzo[*k*]fluoranthene	0.8	0.8	8.6
Benzo[*a*]pyrene	1.4	1.3	5.4
Indeno[1,2,3-*cd*]pyrene	1.3	1.3	8.2
Benzo[*ghi*]perylene	1.5	1.6	8.0

No significant degradation during dispatch even at 60 °C was observed. Therefore, the material can be transported at ambient conditions without special precautions.

The storage temperature of the material is set to 18 °C.

After the certification campaign, the material will be subjected to IRMM's regular stability monitoring programme to control its further stability.

6 Characterisation

The material characterisation is the process of determining the property values of a reference material.

The material characterisation was based on an intercomparison of expert laboratories, i.e. the properties of the material were determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Twelve laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of PAH measurements in water matrices by submitting results of intercomparison exercises or method validation reports. Having a formal accreditation was not mandatory, but meeting the requirements of ISO/IEC 17025 [3] was obligatory. Where measurements are covered by the

scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Each laboratory received three ERM-CA100 box set units and was requested to strictly follow the reconstitution protocol which was provided as well, to obtain the final CRM. Each laboratory was requested to provide six independent results, two per CRM unit. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over at least two days to ensure intermediate precision conditions. An independent calibration was performed for each day. Results were reported as ng/L.

Each participant was asked to analyse the PAH ampoule solution as a blind quality control (QC) sample. The results for this sample were used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed, i.e. top-down and bottom-up were regarded as equally valid procedures.

6.3 Methods used

Analysis methods with different sample pre-treatment [LLE & solid phase extraction (SPE)] and different quantification methods (HPLC-FLD and GC-MS) were used to characterise the material. The combination of results from methods based on completely different principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex D. The laboratory code (e.g. L01) is a random number and does not correspond to the order of laboratories in Section 2. The lab-method code consists of a number assigned to each laboratory (e.g. L01) and the abbreviation of the measurement method used (e.g. L01-GC-MS).

6.4 Evaluation of results

The characterisation campaign resulted in twelve datasets per each PAH. All individual results of the participants, grouped per analyte, are displayed in tabular form in Annex E.

6.4.1 Technical evaluation

The obtained data were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements, as well as analytical sequence determination performed on two days.
- absence of values given as below limit of detection or below limit of quantification
- method performance, i.e.

  agreement of the measurement results with the assigned value of the QC sample. Values reported for the analysis of the QC sample were expected to remain within a 20 % offset from the assigned value (theoretical value of each analyte of the PAH solution B, see Table 1)

  agreement of the mean measurement result with the theoretical values of PAHs in the reconstituted water sample (as obtained from calculating the concentration in water upon spiking). Values reported were accepted upon agreement ± 30 %

from the theoretical value, taking into consideration the expanded uncertainty reported by the laboratories.

Based on the above criteria, the following datasets were rejected as not technically valid (see also Table 7).

L01 and L02: the datasets of benzo[*b*]fluoranthene and benzo[*ghi*]perylene were rejected because the measurement results of the QC sample were deviating more than 20 % from the theoretical QC value.

L03: the datasets of anthracene and benzo[*ghi*]perylene were rejected because the measurement results of the QC sample were deviating more than 20 % from the theoretical QC value.

L04: the datasets of all analytes were rejected because no internal standard was used, consequently the loss of PAHs adsorbed onto the HA could not be compensated for (as reported by the laboratory itself); therefore, the measurements' accuracy was seriously compromised.

L05: the dataset of anthracene was rejected because not fulfilling the agreement of ± 30 % of the theoretical value in the reconstituted water sample.

L06: the datasets of all analytes except anthracene were rejected because the measurement results of the QC sample were deviating more than 20 % from the theoretical QC value.

L08: the datasets of naphthalene, anthracene, benzo[*k*]fluoranthene and benzo[*ghi*]perylene were rejected because the measurement results of the QC sample were deviating more than 20 % from the theoretical QC value; the datasets of benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene were rejected because not fulfilling the agreement of ± 30 % of the theoretical value in the reconstituted water sample.

L09: the datasets of benzo[*ghi*]perylene was rejected because the measurement result of the QC sample was deviating more than 20 % from the theoretical QC value.

L10: the dataset of benzo[*ghi*]perylene was rejected because the measurement result of the QC sample was deviating more than 20 % from the theoretical QC value; the datasets of benzo[*b*]fluoranthene, benzo[*k*]fluoranthene benzo[*a*]pyrene and indeno[1,2,3-*cd*]pyrene were rejected because not fulfilling the agreement of ± 30 % of the theoretical value in the reconstituted water sample.

L11: the datasets of benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene were rejected because not fulfilling the agreement of ± 30 % of the theoretical value in the reconstituted water sample.

L12: the datasets of naphthalene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene were rejected because the measurement results of the QC sample were deviating more than 20 % from the theoretical QC value.

Table 7: Datasets that showed non-compliances with the analysis protocol and technical specifications, and action taken

Analyte	Laboratory code	Description of problem	Action taken
All	L04	No internal standard used	Not used for evaluation
Naphthalene	L06, L08, L12	No agreement with QC sample value	Not used for evaluation
Anthracene	L03, L08	No agreement with QC sample value	Not used for evaluation
	L05	No agreement with the reconstituted water sample value	
Fluoranthene	L06	No agreement with QC sample value	Not used for evaluation
Benzo[*b*]fluoranthene	L01, L02 L06	No agreement with QC sample value	Not used for evaluation
	L10	No agreement with the reconstituted water sample value	
Benzo[*k*]fluoranthene	L06, L08	No agreement with QC sample value	Not used for evaluation
	L10	No agreement with the reconstituted water sample value	
Benzo[*a*]pyrene	L06	No agreement with QC sample value	Not used for evaluation
	L08, L10, L11	No agreement with the reconstituted water sample value	
Indeno[1,2,3-*cd*]pyrene	L06, L12	No agreement with QC sample value	Not used for evaluation
	L08, L10, L11	No agreement with the reconstituted water sample value	
Benzo[*ghi*]perylene	L01, L02, L03, L06, L08, L09, L10, L12	No agreement with QC sample value	Not certified (insufficient number of datasets)
	L11	No agreement with the reconstituted water sample value	

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests and normal probability plots and were tested for

outlying means using the Grubbs test and using the Cochran test for outlying standard deviations (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 8.

Table 8: Statistical evaluation of the technically accepted datasets for ERM-CA100. *p*: number of technically valid datasets

Analyte	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [ng/L]	*s* [ng/L]	$s_{between}$ [ng/L]	s_{within} [ng/L]
Naphthalene	8	No	Yes (L03, L09, L10, L11)	Yes	1210.893	139.731	137.633	59.079
Anthracene	8	No	Yes (L06)	Yes	91.155	12.178	12.008	4.974
Fluoranthene	10	No	Yes (L02)	Yes	103.526	12.564	12.480	3.554
Benzo[*b*]fluoranthene	7	No	Yes (L03)	Yes	32.030	7.420	7.294	3.333
Benzo[*k*]fluoranthene	8	No	Yes (L05)	Yes	37.722	6.427	6.320	2.856
Benzo[*a*]pyrene	7	No	No	Yes	42.172	6.204	6.022	3.647
Indeno[1,2,3-*cd*]pyrene	6	No	Yes (L05)	insufficient data	28.819	4.545	4.365	3.102
Benzo[*ghi*]perylene	2	insufficient data	No	insufficient data	31.425	4.820	4.373	4.964

The laboratory means follow normal distributions. For benzo[*ghi*]perylene, the number of technically accepted datasets is insufficient for a meaningful assessment.

None of the data contains outlying means.

The statistical evaluation flags laboratories L02, L03, L05, L06, L09, L10 and L11 as outlying variances for at least one of the analytes according to Table 8. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (Table 9).

Table 9: Uncertainty of characterisation for ERM-CA100

Analyte	*p*	Mean [ng/L]	*s* [ng/L]	u_{char} [ng/L]
Naphthalene	8	1210.893	139.731	49.402
Anthracene	8	91.155	12.178	4.306
Fluoranthene	10	103.526	12.564	3.973
Benzo[*b*]fluoranthene	7	32.030	7.420	2.805
Benzo[*k*]fluoranthene	8	37.722	6.427	2.272
Benzo[*a*]pyrene	7	42.172	6.204	2.345
Indeno[1,2,3-*cd*]pyrene	6	28.819	4.545	1.856
Benzo[*ghi*]perylene	2	31.425	4.820	3.408

7 Value Assignment

Certified and additional material information values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at IRMM require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the ISO/IEC Guide 98-3 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

Additional material information refers to values based for example on results from only one or two laboratories or in cases where individual measurement uncertainty is high.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 9 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5). The uncertainty related to degradation during transport (u_{sts}) was found to be negligible. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM,\,rel}$) with a coverage factor k given as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{char,rel}^2 + u_{bb,rel}^2 + u_{lts,rel}^2} \qquad \textbf{Equation 9}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{lts} was estimated as described in Section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor k of 2 was applied to obtain the expanded uncertainties.

In the case of anthracene and fluoranthene, the difference between the certified values and the measured values reported by L02 and L10, respectively, is not covered by the respective measurement uncertainties. A closer investigation shows that the measurement uncertainties reported by L02 and L10 (11 % and 12 %, respectively) are among the smallest reported by all laboratories. In consideration of the latter and of the fact that these laboratories are not spotted as outliers by the statistical evaluation, their measurement uncertainties were considered as probably underestimated and it was decided to adjust them to 20 %, which corresponds approximately to the average measurement uncertainty declared by all laboratories for these two analytes.

For benzo[*ghi*]perylene, given that only two datasets were accepted after the technical evaluation and consequently the statistical assessment was not meaningful, it was decided to provide its value only as additional material information (see Section 7.2).

The certified values and their uncertainties are summarised in Table 10.

Table 10: Certified values and their uncertainties for ERM-CA100

Analyte	Certified value	$u_{char, rel}$ [%]	$u_{bb, rel}$ [%]	$u_{sts, rel}$ [%]	$u_{lts, rel}$ [%]	$U_{CRM, rel}$ [%]	U_{CRM} [1)]
Naphthalene	1.21 µg/L	4.1	2.8	1.0	1.4	10.3	0.13 µg/L
Anthracene	91 ng/L	4.7	2.7	0.8	1.8	11.4	11 ng/L
Fluoranthene	104 ng/L	3.8	1.7	0.5	2.6	9.9	11 ng/L
Benzo[*b*]fluoranthene	32 ng/L	8.8	4.7	2.0	8.0	25.5	9 ng/L
Benzo[*k*]fluoranthene	38 ng/L	6.0	1.9	0.8	8.6	21.3	9 ng/L
Benzo[*a*]pyrene	42 ng/L	5.6	4.2	1.3	5.4	17.6	8 ng/L
Indeno[1,2,3-*cd*]pyrene	29 ng/L	6.4	3.3	1.3	8.2	22.0	7 ng/L

[1)] Expanded (k = 2) and rounded uncertainty

7.2 Additional material information

The data provided in this section should be regarded as informative only on the general composition of the material and cannot be, in any case, used as certified or indicative value.

An additional material information value was assigned for benzo[*ghi*]perylene. The value is the unweighted mean value of the means of two accepted sets of data, each set being obtained in a different laboratory and with a method of determination based on GC-MS (see Table 11).

Table 11: Additional material information value for ERM-CA100

Analyte	Value [ng/L]
Benzo[*ghi*]perylene	31

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

Naphthalene, anthracene, fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene and benzo[*ghi*]perylene are clearly defined analytes. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias. The measurands are therefore structurally defined and independent of the measurement method.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibrants of known purity and specified traceability of their assigned values were used and all relevant input parameters were calibrated. The individual results are therefore traceable to the SI, as it is also confirmed by the agreement among the technically accepted datasets. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Many measurement procedures include one or more steps which select specific (or specific groups) of analytes from the sample for the subsequent whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CLSI Guideline C-53A [16] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

It should be borne in mind that the methods used in the characterisation campaign of ERM-CA100 are methods routinely applied for measuring PAHs in water samples. The agreement of results from different methods demonstrates that ERM-CA100 behaves like a real sample.

ERM-CA100 is obtained joining the three items contained in the CRM box set, i.e. water, HA solution and PAH solution via a reconstitution protocol. Therefore, the analytical behaviour should match as close as possible an environmental surface water sample. For samples with other components, i.e. suspended particulate matter that may result in a different matrix effect, the commutability has to be assessed separately.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The material shall be stored at 18 °C ± 5 °C in the dark.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened units.

9.3 Preparation and use of the material (reconstitution)

The CRM unit contains enough water and spiking solution to prepare two reconstituted samples of 500 mL (approximate concentration of carbon: 0.02 g/L), corresponding to the prescribed minimum sample intake. The two samples shall be prepared at the same time.

To make it ready for use, the material has to be reconstituted according to the following procedure:

The ERM-CA100 unit box set contains:

A bottle with at least 1 L water

An ampoule with at least 24 mL HA spiking solution A (water)

An ampoule with at least 2 mL PAH spiking solution B (acetonitrile)

Recommended glassware (use of glassware should take place in a temperature range that complies with their calibrated volume):

- Volumetric glass flask, volume 500 mL, with glass stopper

- Volumetric glass pipette Class A, volume 1 mL

- Volumetric glass pipette Class A, volume 10 mL

- Volumetric glass pipette Class A, volume 2 mL

Reconstitution protocol:

1. Transfer approximately 450 mL of the water into the 500 mL volumetric glass flask.

2. Shake spiking solution A (humic acids solution) manually for approximately 1 minute.

3. Open the ampoule of spiking solution A and pipette 12 mL into the volumetric glass flask.

4. Shake the volumetric flask manually and gently (also by inversion) for approximately 1 minute.

5. Pipette 1 mL from the spiking solution B (PAH solution) into the volumetric glass flask.

N.B.: the pipette tip ought to be immersed below the water surface (to avoid loss of the most volatile PAHs) inside the volumetric glass flask until solution is delivered (flow out from the pipette by gravity) and slowly pulled back.

6. Mix gently by inversion of the volumetric glass flask for 1 minute.

7. Fill the volumetric glass flask with water up to the calibration mark.

8. Mix gently by inversion of the volumetric glass flask once again for 1 minute to achieve good homogeneity.

9. Leave the solution for about 24 hours in a dark place at + 4 °C ± 3 °C.

10. After this period, mix gently by inversion of the volumetric glass flask once again for 1 minute and proceed with the analysis according to the laboratory procedure within 24 hours.

N.B.: During the operations of mixing of the volumetric glass flask (4, 6, 8, and 10) pay attention not to lose drops of sample (e.g., due to leaking stoppers).

9.4 Minimum sample intake

The minimum sample intake to be used is equal to 500 mL (see Section 9.3).

9.5 Use of the certified value

The main purpose of this material is to assess method performance, i.e. for checking accuracy of analytical results or calibrating analytical procedures. As any reference material, it can be used for establishing control charts or validation studies.

Use as a calibrant

It is not recommended to use this matrix material as calibrant.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1, www.erm-crm.org [17].

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of about 95 %.

Use in quality control charts

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Acknowledgements

The authors would like to acknowledge the support received from members of the Processing Team from IRMM related to the processing of this CRM, from Maria Contreras Lopez concerning the set-up of the required isochronous studies and from Berit Sejeroe-Olsen and Saioa Elordui-Zapatarietxe for laboratory-related matters.

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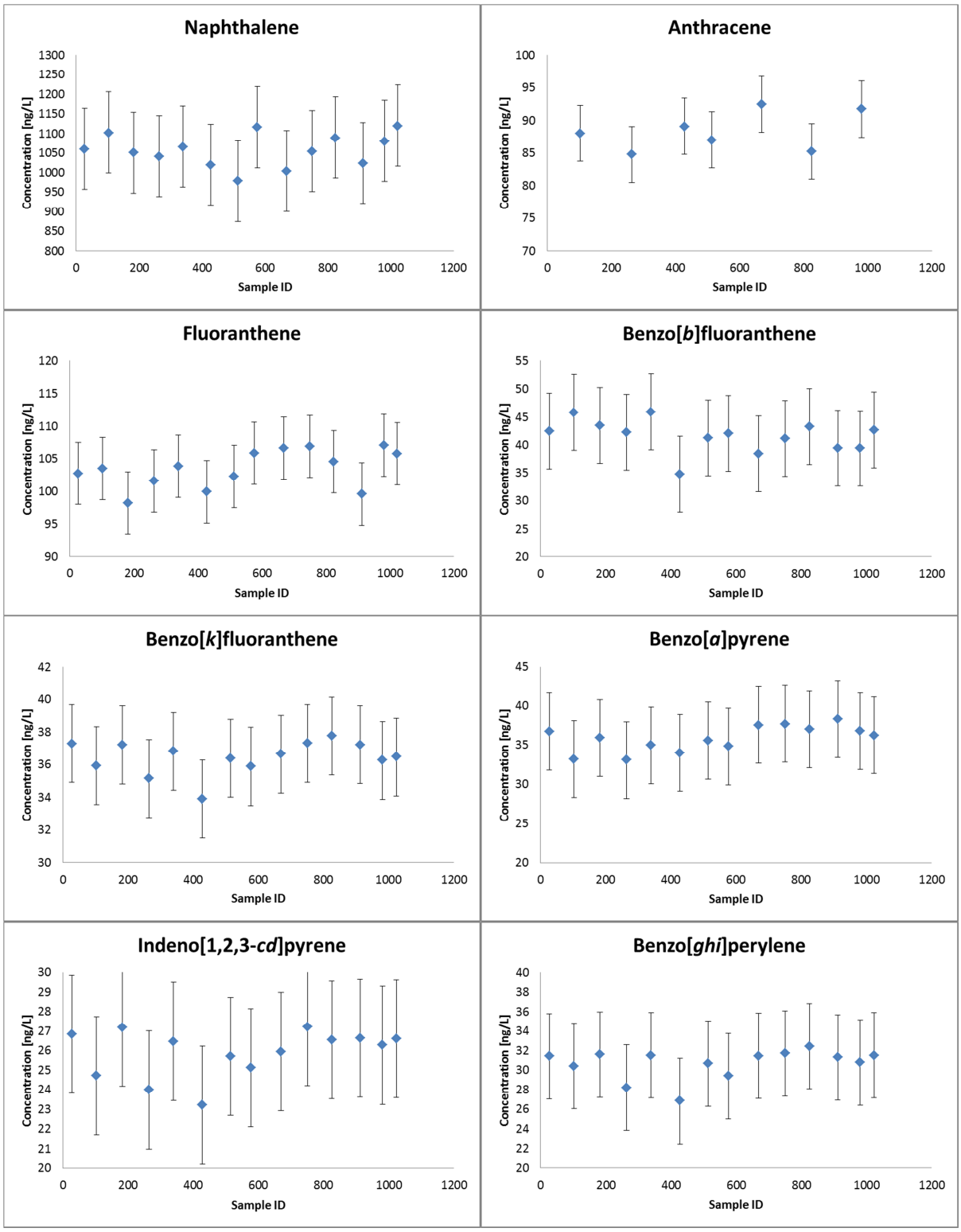
Annexes

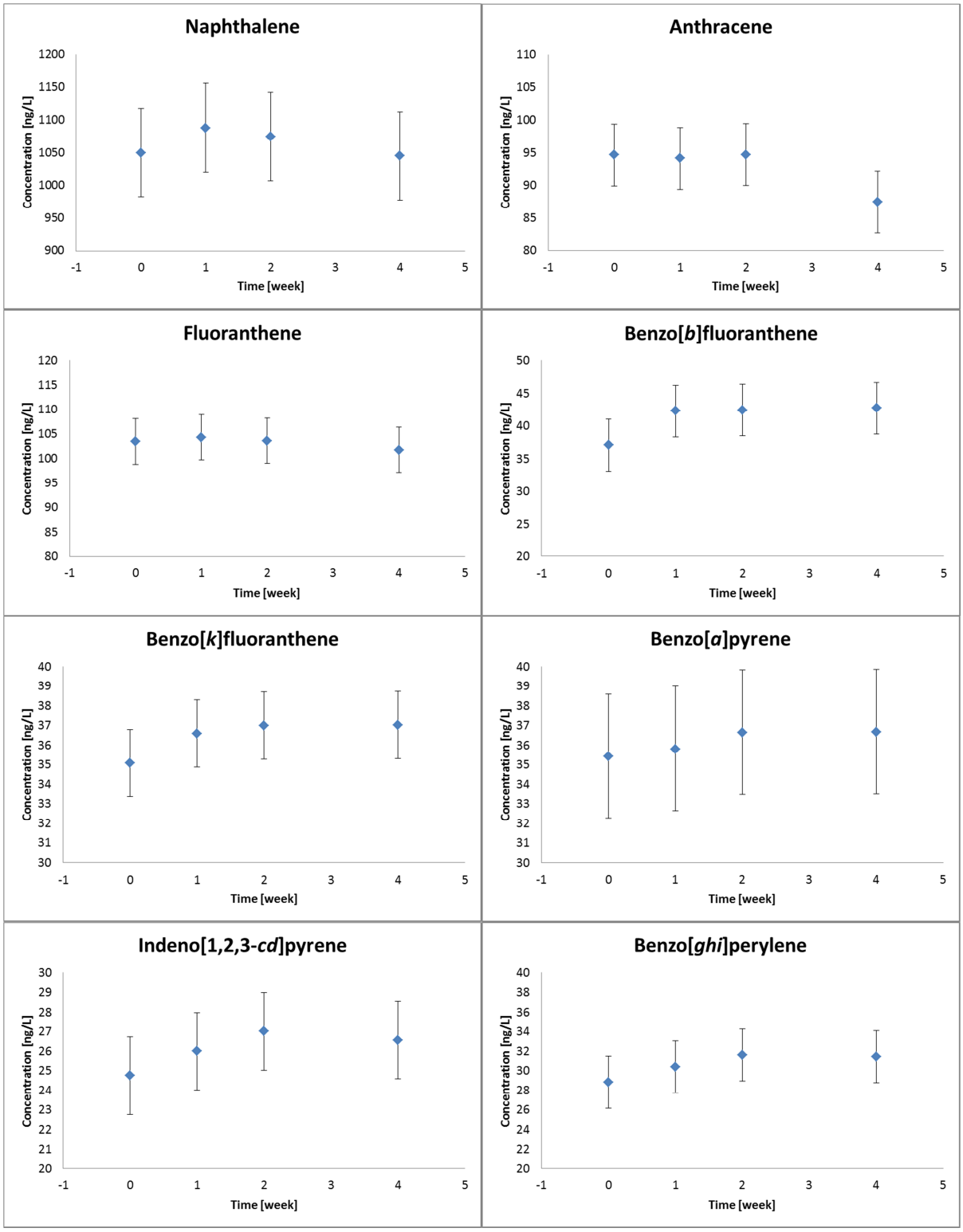
Annex A: Results of the homogeneity measurements

- The graphs report unit means ± 95 % confidence interval (CI) of the means.

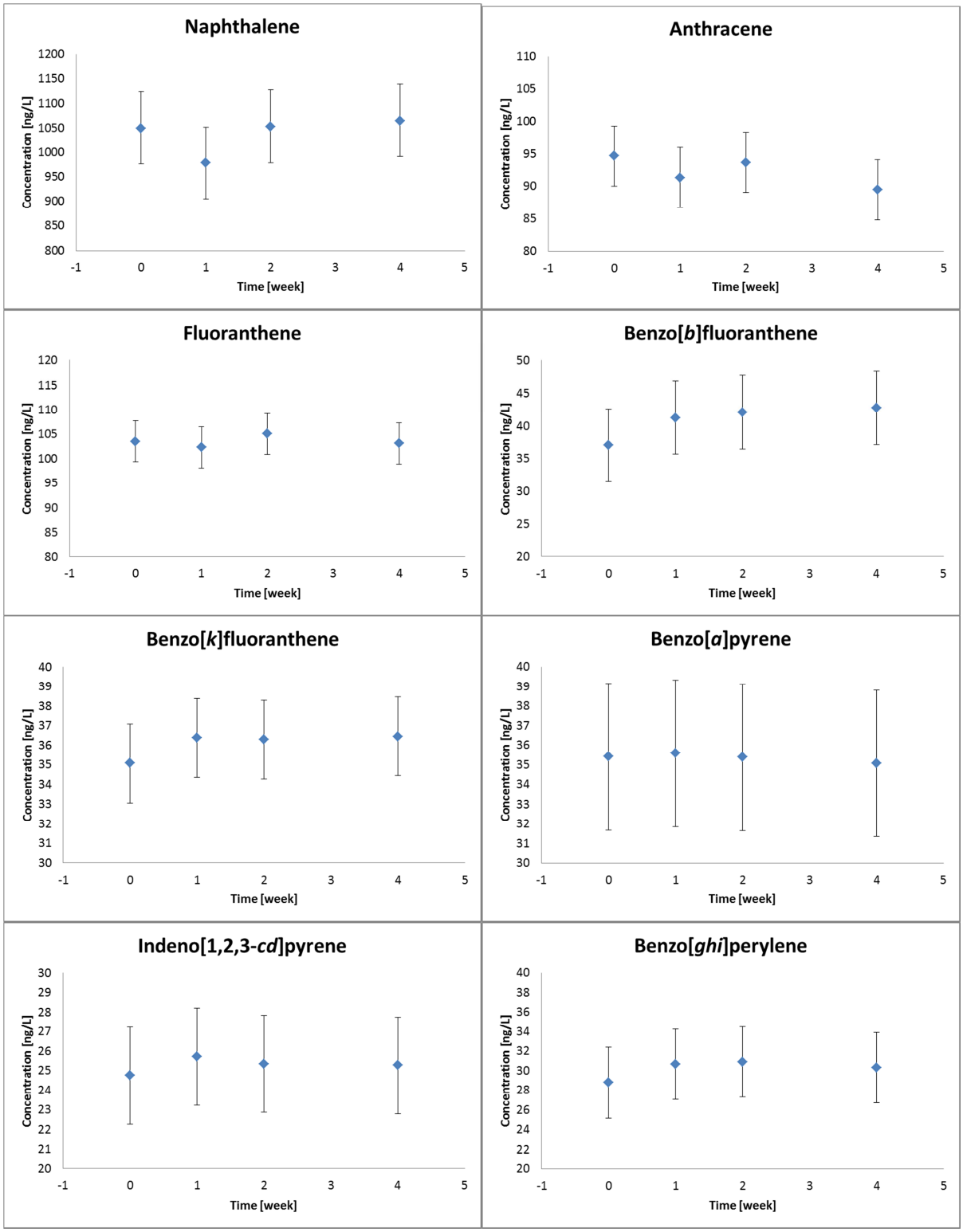


Annex B: Results of the short-term stability measurements

- Data for the short-term stability study at 18 °C. The graphs report means per time point ± 95 % CI of the means.

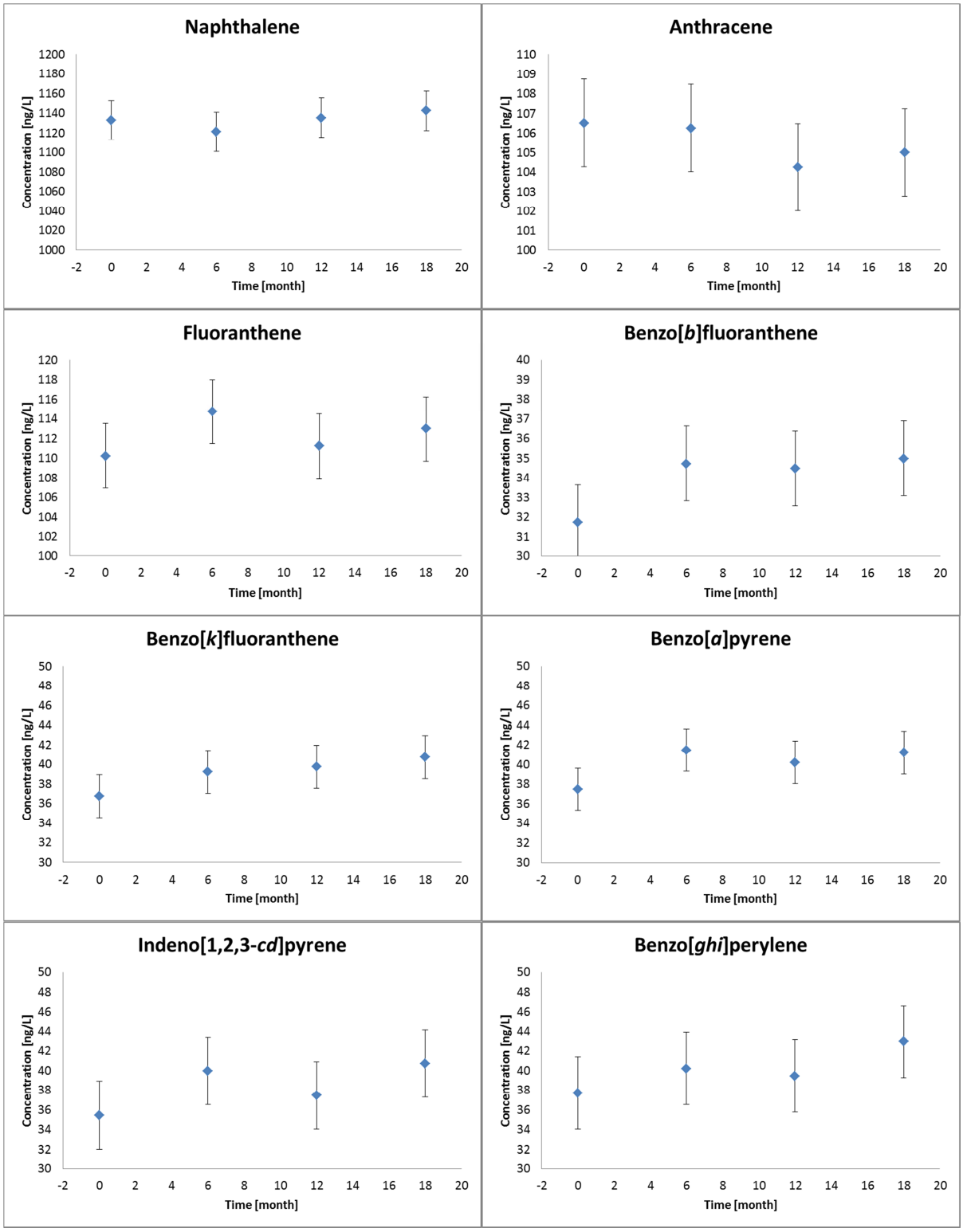


- Data for the short-term stability study at 60 °C. The graphs report means per time point ± 95 % CI of the means.



Annex C: Results of the long-term stability measurements

- Data for the long-term stability study at 18 °C. The graphs report means per time point ± 95 % CI of the means.



Annex D: Summary of methods used in the characterisation study

- Method information is reported as given by laboratories
- All laboratories had to follow the reconstitution protocol to obtain the final CRM

Laboratory code – method	Sample pre-treatment	Analytical method	Type of calibration Calibrants' details	LOQ [ng/L]
L01-GC-MS	LLE (liquid-liquid extraction), extract concentration	GC-MS Labelled PAHs added as internal standard	External: 1000/ 600 / 300 / 100 / 60 / 30 / 10 ng/mL PAH standard mix PAH standard mix, Promochem, DE-Prom16, 100 µg/mL	10
L02-GC-MS	LLE, extract clean-up with silica/alumina column	GC-MS Labelled PAHs added as internal standard	External: 1-point calibration - concentration ca.1 µg/mL NIST 2260A	10
L03-HPLC-FLD	LLE, extract concentration with silica column	HPLC-FLD B-B binaphtyl used as internal standard	External: 0.031 - 480 ng/mL NIST SRM 1647 e, 0.79-19.85 mg/L	0.1–5
L05-GC-MS	SPE (solid phase extraction and clean-up)	GC-MS Labelled PAHs added as internal standard	Internal: 0.5 µg/L in water Accustandard PAH mix 2 mg/mL	10-20
L06-GC-MS	LLE, extract concentration	GC-MS Labelled PAHs added as internal standard.	External: 1, 2, 5, 10, 25, 50, 100, 250, 500, 1000 ng/mL Dr. Ehrenstorfer Mix 9 100 µg/mL cyclohexane, CIL 100 µg/mL nonane	10
L07-GC-MS	LLE, extract concentration	GC-MS Labelled PAHs added as internal standard.	Internal: Naphthalene 500 / 550 / 600 / 650 / 700 ng/mL each; ^{13}C-Naphthalene 600 ng/mL 7 PAHs 5 / 20 / 35 / 50 / 65 ng/mL; ^{13}C-PAHs 35 ng/mL Dr Ehrenstorfer, 16 PAHs in cyclohexane, 10 ng/µL each CIL, 100 µg/mL in *n*-nonane	1-10
L08-GC-MS	SPE extraction and clean-up	GC-MS Labelled PAHs added as internal standard.	Internal: 5-10-25-50-100-250-500 ng/mL PAH-mix 45, Dr Ehrenstorfer 10 ng/µL in ACN	1 -40
L09-GC-MS	LLE, extract concentration	GC-MS Labelled PAHs added as internal standard	External: 3 points calibration curve Accustandard S-21969-R1-100X-5mL	0.07-30
L10-GC-MS	SPE extraction and clean-up	GC-MS Labelled PAHs added as internal standard	External: (repeated one point calibration): 8-200 ng/mL calibration Range: 0-1000 ng/mL extract 16 EPA PAH: NIST SRM1649e 8-200 ng/mL	0.2–4.4
L11-HPLC-FLD	LLE, extract clean-up with silica/alumina column	HPLC-FLD Labelled PAHs added as internal standard	External: 9-point calibration - concentration ca.1 - 500 ng/mL Dr. Ehrenstorfer,(ref. L20950009AL) (CAS:75-05-8) 10 µg/mL	2

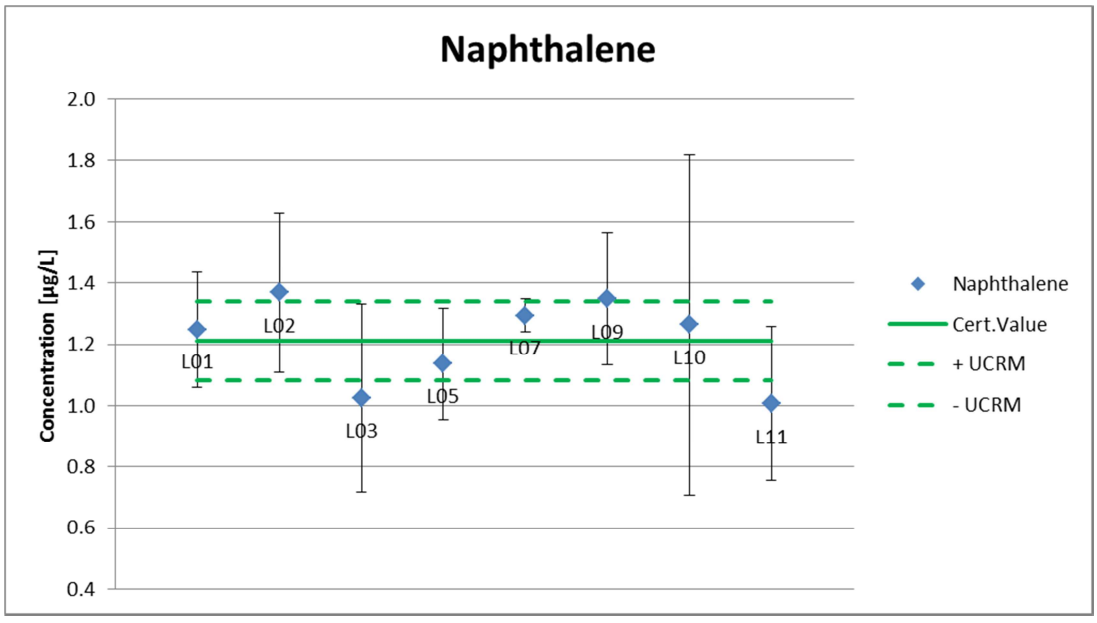
L12-GC-MS	LLE, extract clean-up with silica/alumina column	GC-MS Labelled PAHs added as internal standard	External: 0.0032, 0.016, 0.08, 0.8, 4, 10 µg/mL PAH-Mix, Dr. Ehrenstorfer, original 10 µg/mL, diluted to 1 µg/mL	0.1–3.7
Not used in certification				
L04-LC-APPI-MS/MS	SPE extraction and clean-up	LC-FL quantification LC-APPI-MS/MS confirmation/identification No internal standard	External: 1, 5, 10, 25, 50, 75 & 100 ng/mL except for naphthalene and fluoranthene for which are 10, 50, 100, 250, 500, 750 & 1000 ng/mL A standard solution (S1), mixture of 8 PAHs is prepared at a concentration of 1 µg/mL by mixing appropriate volumes of individual standard solutions of each PAH with concentration of 100 µg/mL	10–100

Annex E: Results of the characterisation measurements

Note: *Reported values were rounded*

Naphthalene

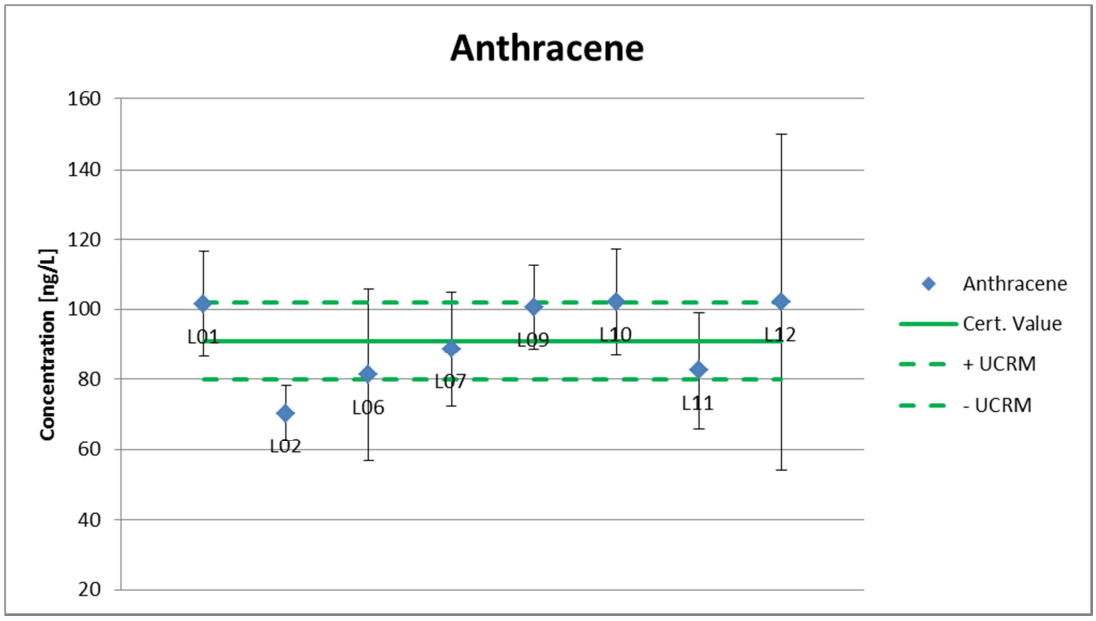
Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	1247	1254	1238	1225	1265	1255	1247	187
L02-GC-MS	1360	1380	1360	1340	1380	1390	1368	260
L03-HPLC-FLD	1068	1004	932	1113	1012	1015	1024	307
L05-GC-MS	1150	1150	1120	1140	1140	1120	1137	182
L07-GC-MS	1292	1282	1294	1296	1293	1301	1293	54
L09-GC-MS	1305	1404	1356	1301	1403	1314	1347	216
L10-GC-MS	1244	1319	1280	1244	1272	1227	1264	556
L11-HPLC-FLD	1036	1114	862	993	1198	834	1006	252
Results not used for certification								
L04-LC-APPI-MS/MS	*1114*	*1139*	*1113*	*1076*	*1126*	*1070*	*1106*	*310*
L06-GC-MS	*1041*	*1059*	*1584*	*1049*	*1044*	*1562*	*1224*	*367*
L08-GC-MS	*1294*	*1357*	*1405*	*1287*	*1348*	*1324*	*1336*	*504*
L12-GC-MS	*1550*	*1420*	*1640*	*1440*	*1380*	*1690*	*1520*	*713*



Anthracene

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	100.7	101.9	101.4	99.8	103.2	102.5	101.6	15.2
L02-GC-MS	62.2	70.4	74.3	71.9	71.4	72.6	70.5	14.1*
L06-GC-MS	73.4	74.3	96.9	75.6	77.4	90.9	81.4	24.4
L07-GC-MS	88.3	90.6	89.3	83	89.9	90.9	88.7	16.1
L09-GC-MS	101.8	99.2	102.5	102.4	100.3	97.2	100.6	12.1
L10-GC-MS	99	106	104	100	98	105	102	15.3
L11-HPLC-FLD	87	84.2	73.6	88.2	87.6	74.4	82.5	16.5
L12-GC-MS	102	102	98.3	105	99	106	102.1	47.9
Results not used for certification								
L03-HPLC-FLD	*91.5*	*85.4*	*92.3*	*93.2*	*92.4*	*92*	*91.1*	*27.3*
L04-LC-APPI-MS/MS	*78*	*80*	*78*	*76*	*80*	*74*	*77.7*	*11.3*
L05-GC-MS	*159*	*142*	*144*	*138*	*149*	*158*	*148.3*	*16.3*
L08-GC-MS	*106.9*	*111.4*	*108.4*	*95*	*111.7*	*103.6*	*106.2*	*31.4*

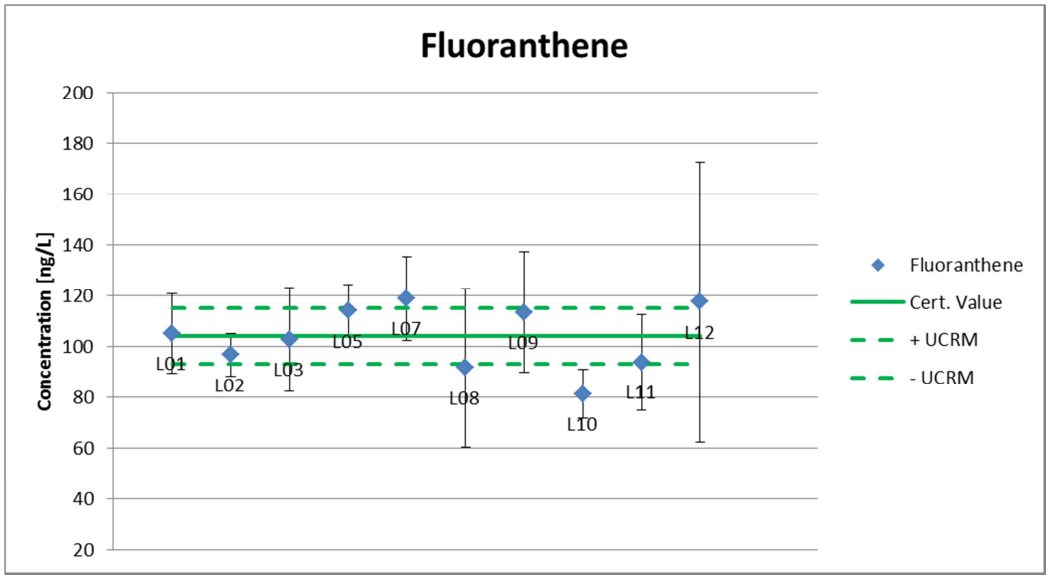
*adjusted from the reported 11 % to 20 % (see Section 7.1)



Fluoranthene

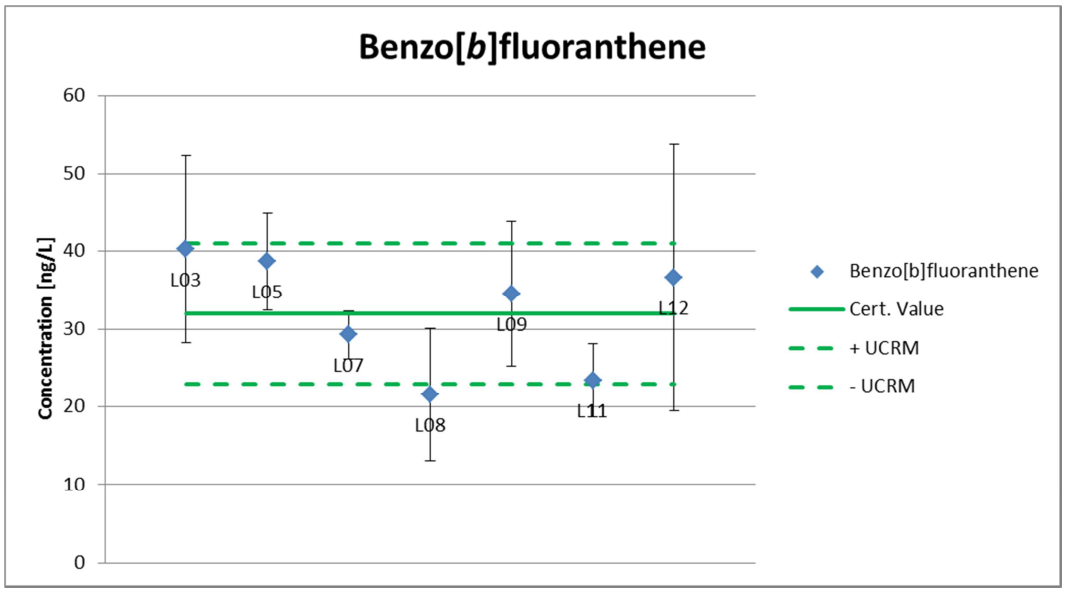
Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	104.8	107.1	104.3	105.2	105.2	104	105.1	15.8
L02-GC-MS	87.1	95.5	103	107	92.8	94.6	96.7	8.4
L03-HPLC-FLD	105.9	98	108.1	99.8	101.8	103	102.8	20.6
L05-GC-MS	121	113	111	113	111	115	114	10.3
L07-GC-MS	117.9	118	121.6	116.7	119.2	120.4	119	16.5
L08-GC-MS	90.52	94.38	89.04	94.14	92.76	89.22	91.7	31.3
L09-GC-MS	110.1	116	115	111	115.6	113.2	113.5	23.8
L10-GC-MS	80	78	83	81	80	86	81.3	16.3*
L11-HPLC-FLD	91.2	90.2	98	93.4	93	96.8	93.8	18.8
L12-GC-MS	122	115	122	114	116	116	117.5	55.1
Results not used for certification								
L04-LC-APPI-MS/MS	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	-
L06-GC-MS	*73.8*	*73.9*	*78.6*	*76.7*	*71.5*	*89.4*	*77.3*	*23.2*

*adjusted from the reported 12 % to 20 % (see Section 7.1)



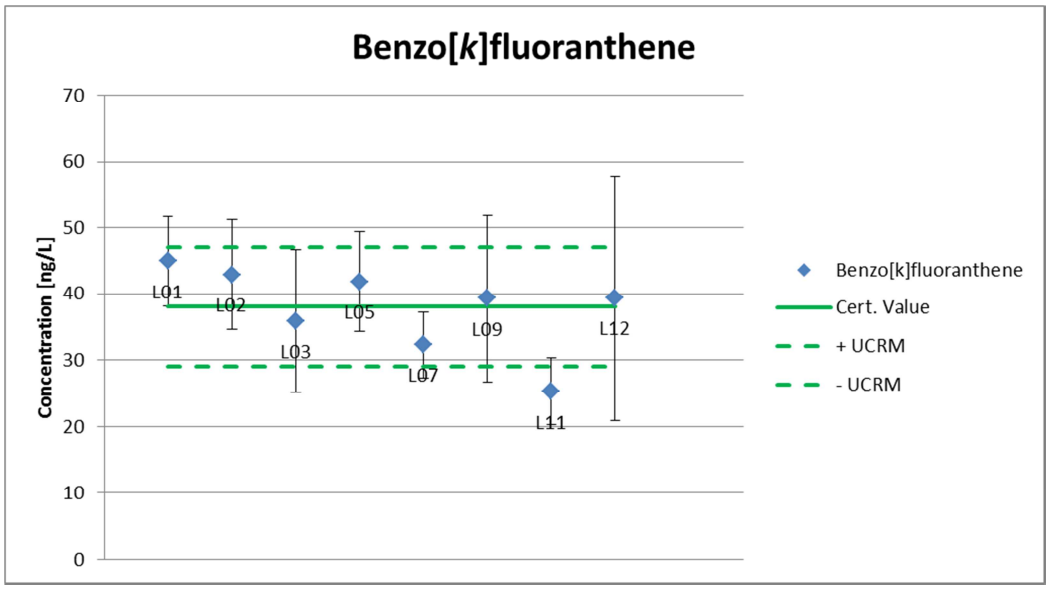
Benzo[*b*]fluoranthene

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L03-HPLC-FLD	45.7	42	36.7	34.3	34.7	48.2	40.3	12.1
L05-GC-MS	48	35	36	40	37	36	38.7	6.2
L07-GC-MS	27	29.9	30.1	29.2	29	30.3	29.3	3.1
L08-GC-MS	22.1	23.2	17	24.2	22.7	20.4	21.6	8.5
L09-GC-MS	33.7	34	35.5	33.8	35	34.9	34.5	9.3
L11-HPLC-FLD	23	24	20.4	25.2	25.6	22	23.4	4.7
L12-GC-MS	36	41.1	35.9	36.7	36.4	33.4	36.6	17.2
Results not used for certification								
L01-GC-MS	*37.7*	*37.2*	*36.8*	*36.2*	*38.6*	*37.9*	*37.4*	*5.6*
L02-GC-MS	*25.6*	*23.6*	*30.1*	*26*	*22.3*	*26.7*	*25.7*	*1.5*
L04-LC-APPI-MS/MS	*10*	*10*	*10*	*<LOQ*	*10*	*<LOQ*	*10*	*2.5*
L06-GC-MS	*10.8*	*14.4*	*<LOQ*	*14.1*	*<LOQ*	*11.2*	*12.7*	*3.8*
L10-GC-MS	*17*	*16*	*20*	*18*	*20*	*23*	*19*	*2.7*



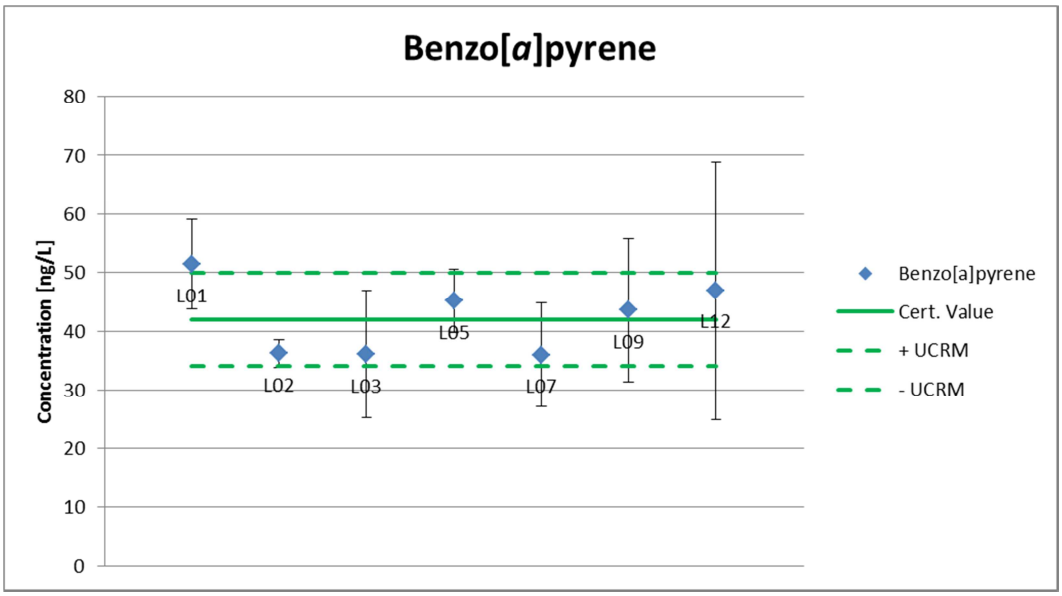
Benzo[*k*]fluoranthene

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	44.9	44	43.7	44.1	47.1	45.8	44.9	6.7
L02-GC-MS	45.7	39.4	40.7	46.5	39.8	45.5	42.9	8.4
L03-HPLC-FLD	34.4	34.4	37.5	36.7	36.5	35.7	35.9	10.8
L05-GC-MS	53	40	38	45	38	37	41.8	7.5
L07-GC-MS	30.9	33.6	34.5	30.9	31.5	32.4	32.3	4.9
L09-GC-MS	38.72	38.69	40.34	38.9	39.84	39.25	39.3	12.6
L11-HPLC-FLD	25.2	26.4	22	26.8	27.4	23.8	25.3	5.1
L12-GC-MS	37.9	43.2	39.4	39.8	40	35.8	39.4	18.5
Results not used for certification								
L04-LC-APPI-MS/MS	*11*	*11*	*11*	*10*	*11*	*10*	*10.7*	*1.6*
L06-GC-MS	*12.13*	*15.05*	*14.92*	*14.72*	*12.39*	*15.88*	*14.2*	*4.3*
L08-GC-MS	*25.98*	*25.12*	*20.08*	*28.52*	*24.52*	*26.1*	*25.1*	*5.9*
L10-GC-MS	*15*	*19*	*17*	*15*	*22*	*21*	*18.2*	*3.1*



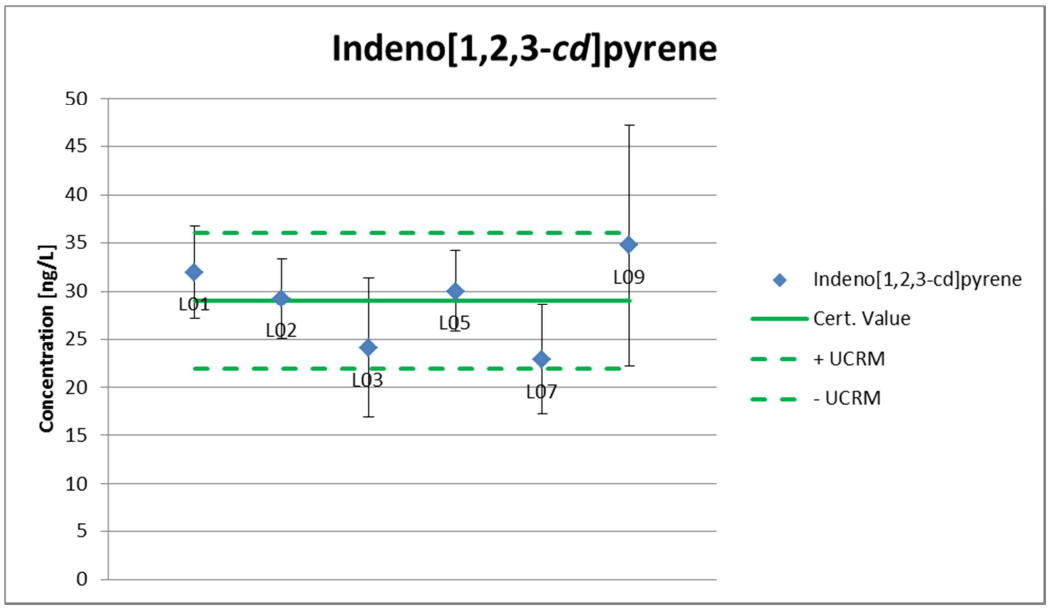
Benzo[*a*]pyrene

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	51.8	51.7	50.5	49.5	52.9	52	51.4	7.7
L02-GC-MS	40.9	37.5	31.3	36.1	38.6	32.6	36.2	2.4
L03-HPLC-FLD	30.7	34.1	40.1	39.6	39.4	32.4	36.1	10.8
L05-GC-MS	56	42	41	48	42	42	45.2	5.4
L07-GC-MS	34.3	37.9	40.6	31.1	35.7	36.1	36	8.8
L09-GC-MS	42.3	44.1	45.8	41.7	44.3	43	43.5	12.2
L12-GC-MS	46.6	53.8	47.1	44.8	46.6	42.7	46.9	22.0
Results not used for certification								
L04-LC-APPI-MS/MS	*11*	*12*	*11*	*10*	*12*	*10*	*11*	*1.9*
L06-GC-MS	*15.83*	*17.36*	*15.26*	*17.8*	*16.13*	*17.5*	*16.7*	*5*
L08-GC-MS	*26.7*	*29.1*	*19.1*	*27.1*	*26.1*	*24.8*	*25.5*	*9.0*
L10-GC-MS	*15*	*18*	*18*	*17*	*23*	*21*	*18.7*	*3.0*
L11-HPLC-FLD	*28*	*29.4*	*25.2*	*29.8*	*31*	*27*	*28.4*	*5.7*



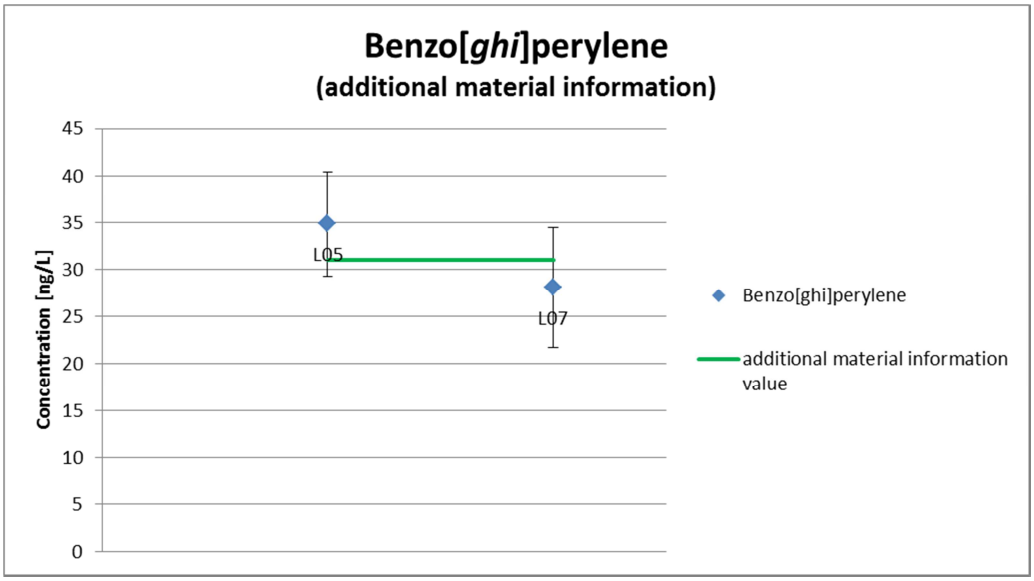
Indeno[1,2,3-*cd*]pyrene

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L01-GC-MS	30.3	32.5	31.8	31.6	32.9	32.7	32	4.8
L02-GC-MS	25.8	29.5	27	35.3	29.4	28.1	29.2	4.1
L03-HPLC-FLD	23.2	21.8	25.9	24.5	25	24.3	24.1	7.2
L05-GC-MS	41	26	26	33	28	26	30	4.2
L07-GC-MS	21.2	27.4	20.7	22.4	24.4	21.4	22.9	5.7
L09-GC-MS	34.3	33.7	36	33.9	35.2	35.3	34.7	12.5
Results not used for certification								
L04-LC-APPI-MS/MS	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	-
L06-GC-MS	*<LOQ*	*10.16*	*<LOQ*	*11*	*<LOQ*	*<LOQ*	*10.6*	*3.2*
L08-GC-MS	*19.7*	*17.7*	*12.7*	*18.6*	*16.3*	*15.1*	*16.7*	*6.7*
L10-GC-MS	*10*	*14*	*12*	*14*	*17*	*17*	*14*	*2.0*
L11-HPLC-FLD	*19.6*	*18.4*	*15.6*	*18.2*	*19.6*	*16.2*	*17.9*	*3.6*
L12-GC-MS	*33.6*	*43.6*	*38.4*	*31.8*	*38.6*	*33.5*	*36.6*	*17.2*



Benzo[*ghi*]perylene (additional material information)

Laboratory code	replicate 1 [ng/L]	replicate 2 [ng/L]	replicate 3 [ng/L]	replicate 4 [ng/L]	replicate 5 [ng/L]	replicate 6 [ng/L]	mean [ng/L]	Expanded uncertainty [ng/L]
L05-GC-MS	47	30	31	38	32	31	34.8	5.6
L07-GC-MS	28.3	29.1	28.7	23.8	27.4	30.8	28.0	6.4
Results not used for certification								
L01-GC-MS	*34.7*	*38.1*	*37.6*	*37.1*	*39.6*	*37.6*	*37.5*	*5.6*
L02-GC-MS	*34.3*	*35.9*	*31.5*	*33.9*	*37.9*	*32.9*	*34.4*	*5.8*
L03-HPLC-FLD	*27.9*	*27*	*31.9*	*30.4*	*30.9*	*29.7*	*29.6*	*11.9*
L04-LC-APPI-MS/MS	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	-
L06-GC-MS	*11.78*	*16.01*	*8.35*	*15.75*	*12.09*	*11*	*12.5*	*3.8*
L08-GC-MS	*19.7*	*23.2*	*16.1*	*18.5*	*17.4*	*20.9*	*19.3*	*6.5*
L09-GC-MS	*44.5*	*45.6*	*49.2*	*44.3*	*47.3*	*48.4*	*46.5*	*12.1*
L10-GC-MS	*13*	*18*	*18*	*17*	*24*	*25*	*19.2*	*2.5*
L11-HPLC-FLD	*23.8*	*25*	*21*	*24.8*	*26.4*	*22.6*	*23.9*	*4.8*
L12-GC-MS	*40.7*	*54*	*47.8*	*40.8*	*47.3*	*41.9*	*45.4*	*21.3*



European Commission

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